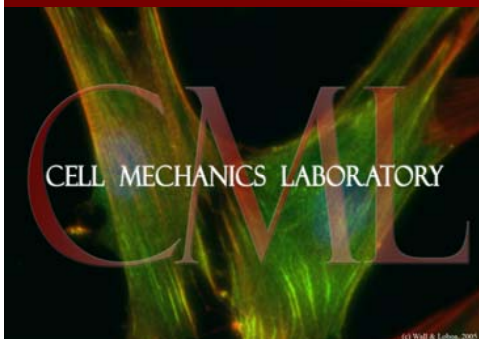


Effects of Cyclic Hydrostatic Pressure on Chondrogenesis of Human Adipose-Derived Adult Stem Cells

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Purpose

- Cartilage injuries
 - Limit joint movement
 - Cause joint pain
- Stem Cells show promise for treatment
 - Differentiate with chemical stimuli and CHP^{1,2}
 - Adipose stem cells have benefits over Bone Marrow
 - Procedure for harvest is less invasive than bone marrow
 - Routinely available for research from liposuctions and surgeries
 - Larger supply of cells available
 - Previous studies with chemical stimuli showed conflicting results for the chondrogenic potential of hMSCs compared to hASCs^{3,4}
- But how do stem cells react to CHP alone?

(1) Mackay, A.M. et al. Tissue Eng. 4, 415, 1998. (2) Yoo, J.U. et al. J. Bone Joint Surg. Am. 80, 1745, 1998.
(3) De Ugarte, Daniel A. et al. Cells Tissue Organs; 174: 101-109 (4) Gun-Il Im M.D. et al. OsteoArthritis and Cartilage; 13: 845-853

Previous Studies

- CHP has been shown to increase expression of genes associated with chondrogenesis in hMSCs in the presence of chemical stimuli^{5,6,7}
- Two previous studies performed in our lab used CHP alone to induce chondrogenesis⁸
 - Bone Marrow derived hMSCs
 - Trabecular Bone derived hMSCs
- Same experimental set up and same RT-PCR procedure
 - Initial increase in Sox9 mRNA expression suggests early chondrogenesis
 - Suggests that CHP is capable of inducing chondrogenic differentiation

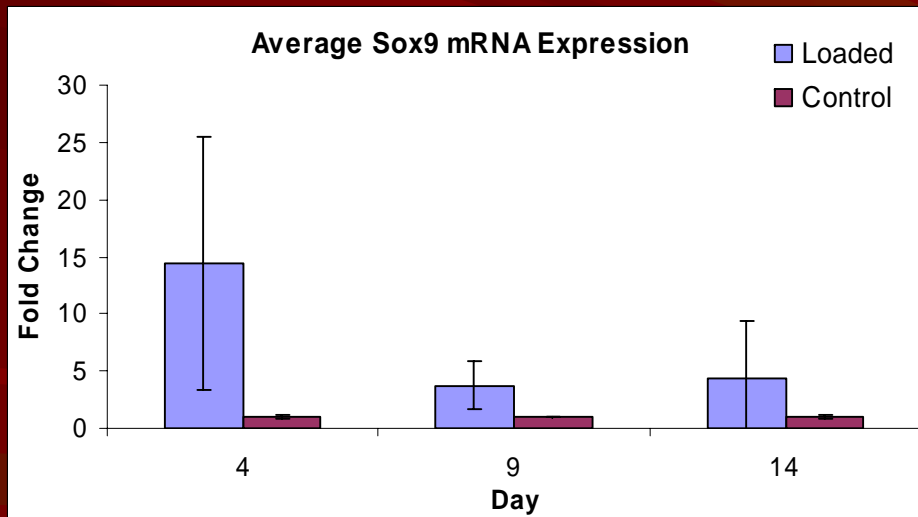


Figure 1: Average Sox9 mRNA expression for bone marrow derived hMSCs
(* indicates $p < 0.05$)

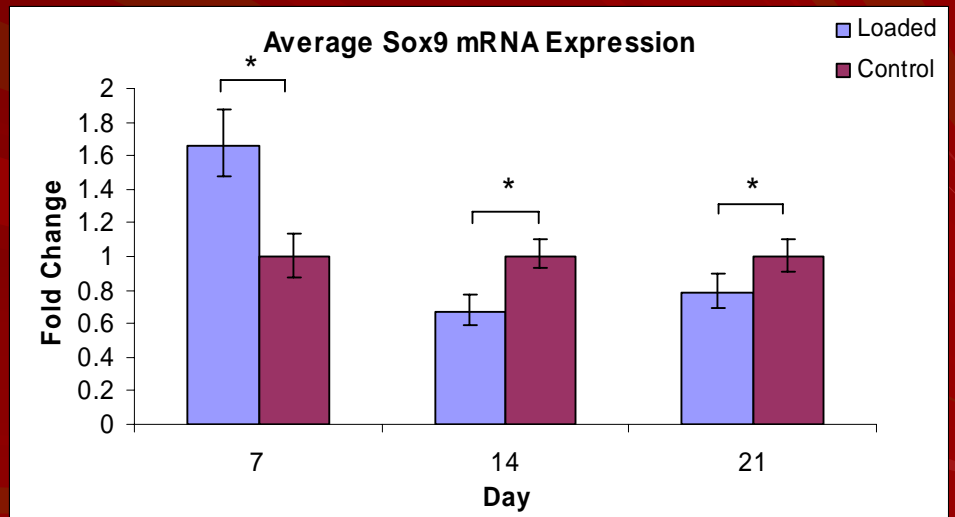


Figure 2: Average Sox9 mRNA expression for trabecular bone derived hMSCs
(* indicates $p < 0.05$)

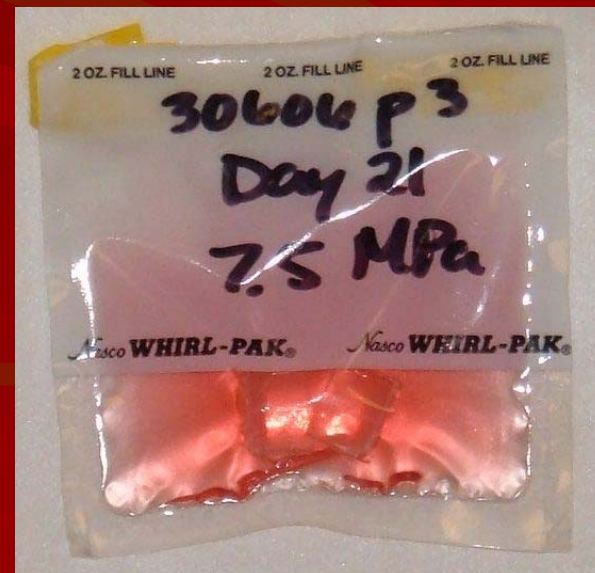
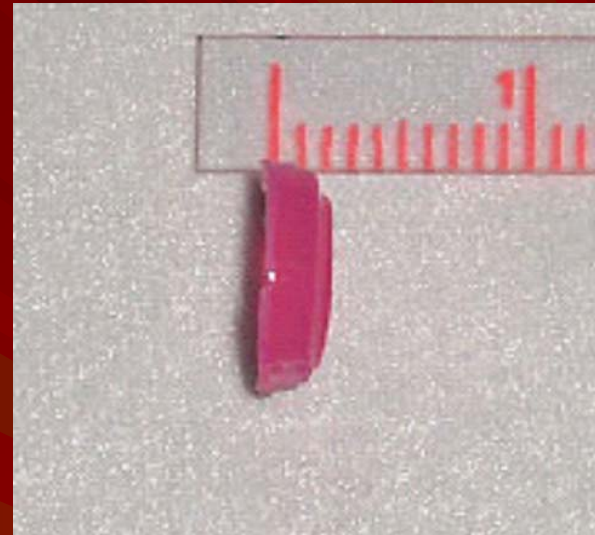
(5) Angele, P. et al. J. Orthop. Res. 21, 451, 2003 (6) Miyanishi, K. et al. Tissue Eng. 12, 2253, 2006
(7) Miyanishi, K. et al. ORS, 2004, abstract 836 (8) Finger, A. et al. Tissue Eng. 13, 1151-1158, 2007

Hypotheses

- Human ASCs would initiate chondrogenic differentiation in response to cyclic hydrostatic pressure
 - In the absence of chondrogenic media
 - Based on previous study which demonstrated hMSCs do differentiate with CHP
- Chondrogenic markers would increase with increased duration of CHP

Methods

- 2 donors
- Seeded in constructs
- Constructs put in heat sealed bags
- Run at 7.5MPa for 4 hrs/day at 1Hz powered by an MTS 858 Mini Bionix II load frame
- In a 1L oil filled Pressure Vessel



MTS Mini
Bionix
Load Frame

MTS

858 Mini Bionix-II

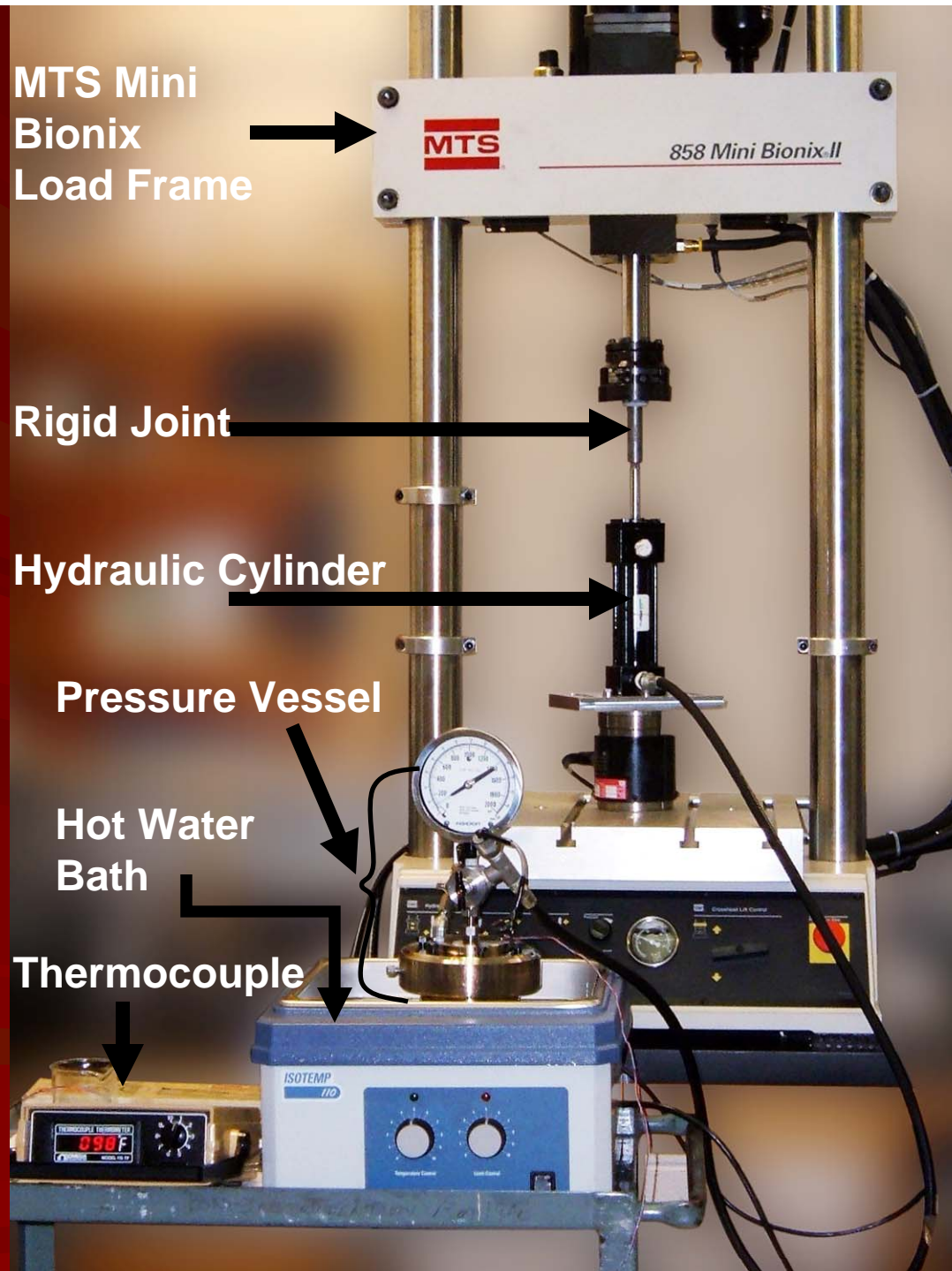
Rigid Joint

Hydraulic Cylinder

Pressure Vessel

Hot Water
Bath

Thermocouple



Real Time RT - PCR

- Analysis on samples from Days 0, 7, 14, and 21
- Total RNA was isolated from each construct, quantified, and reverse transcribed into cDNA
- RT-PCR performed for
 - Aggrecan
 - Sox9
 - COMP
 - Collagen II
- mRNA levels normalized to GAPDH
- Statistical analyses done with Student's t-tests with p-values less than 0.05 considered significant

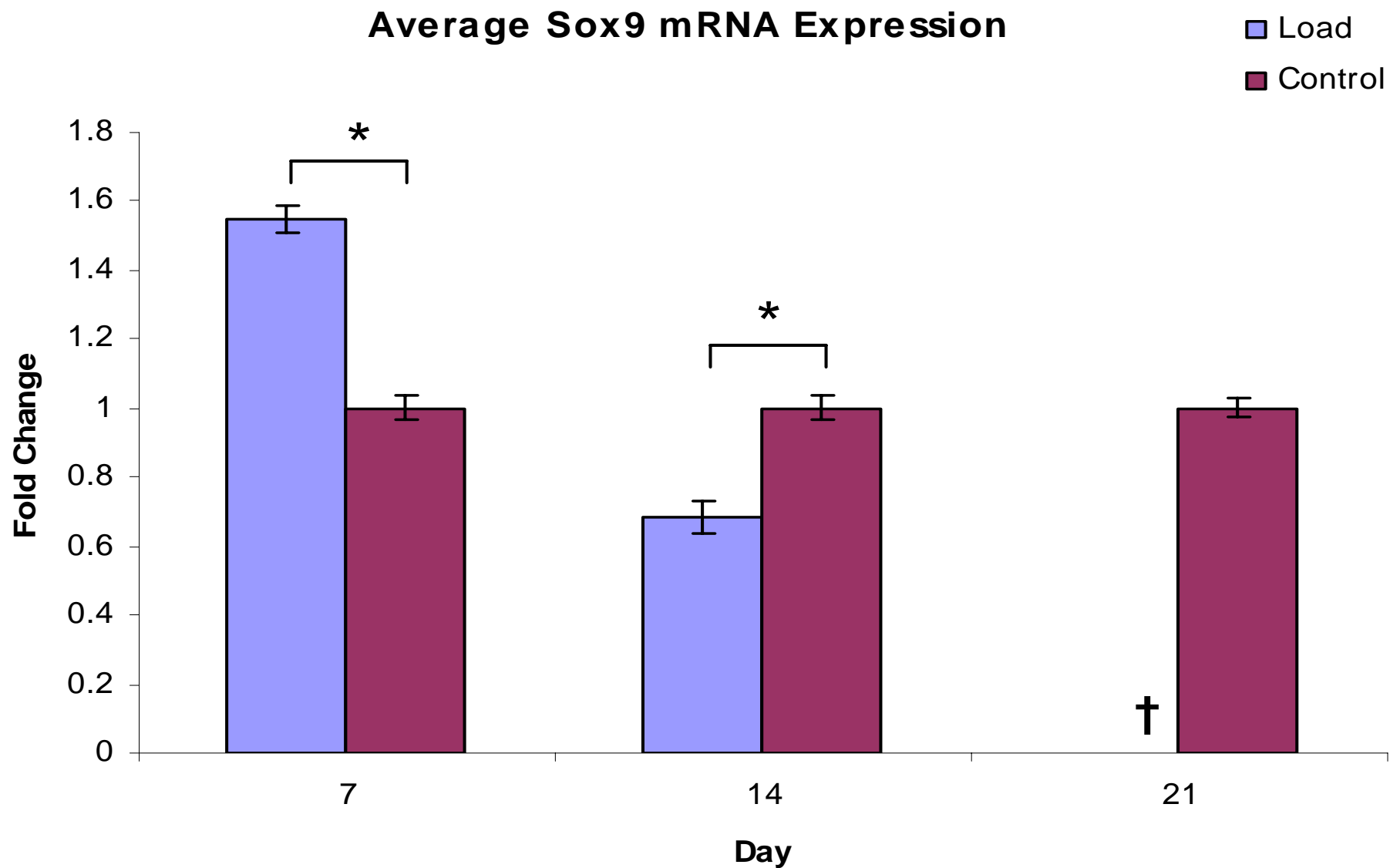


Figure 1: Average Sox9 mRNA expression (* indicates $p < 0.05$ and † indicates no data due to low GAPDH values)

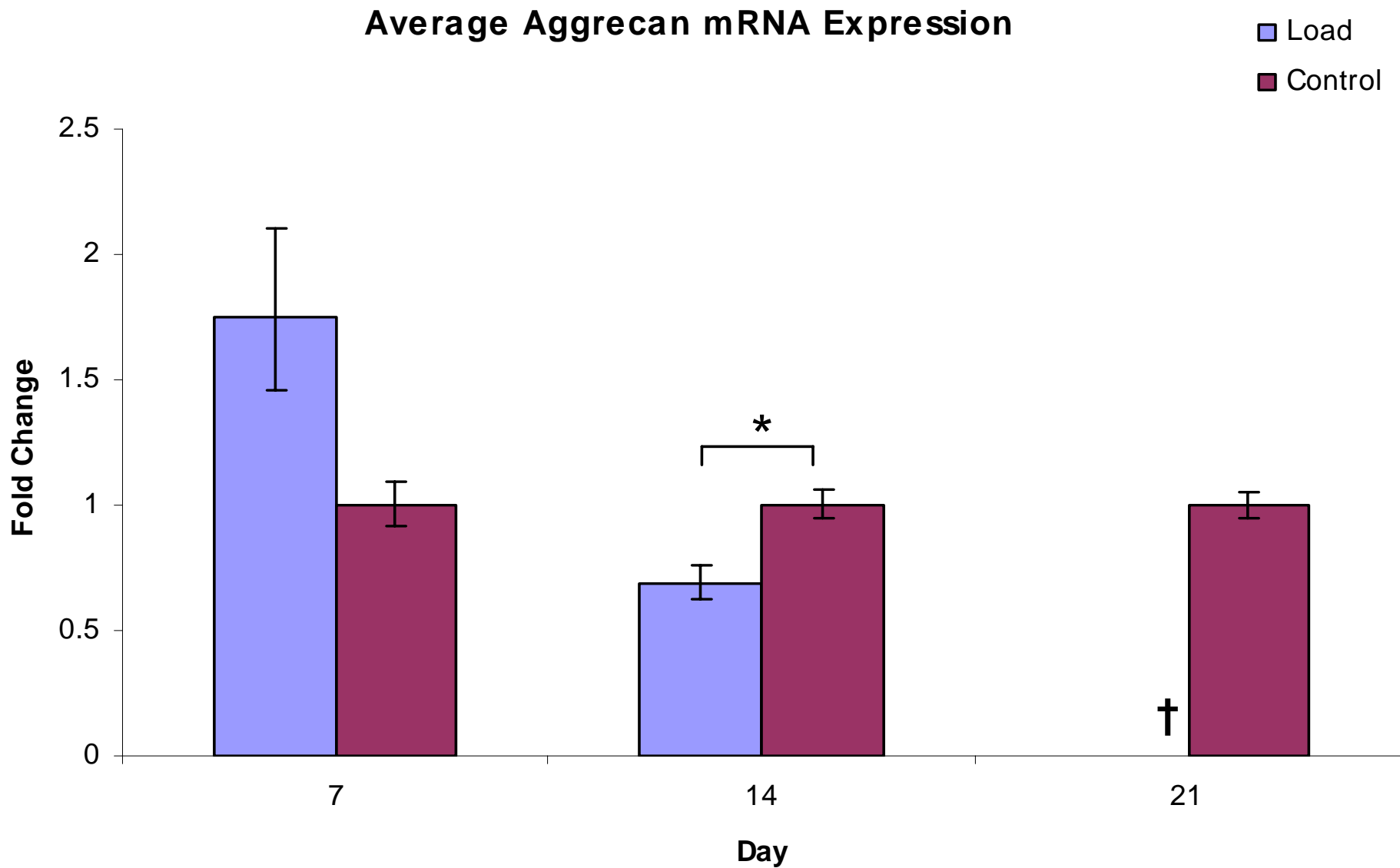


Figure 2: Average Aggrecan mRNA expression (* indicates $p < 0.05$ and † indicates no data due to low GAPDH values)

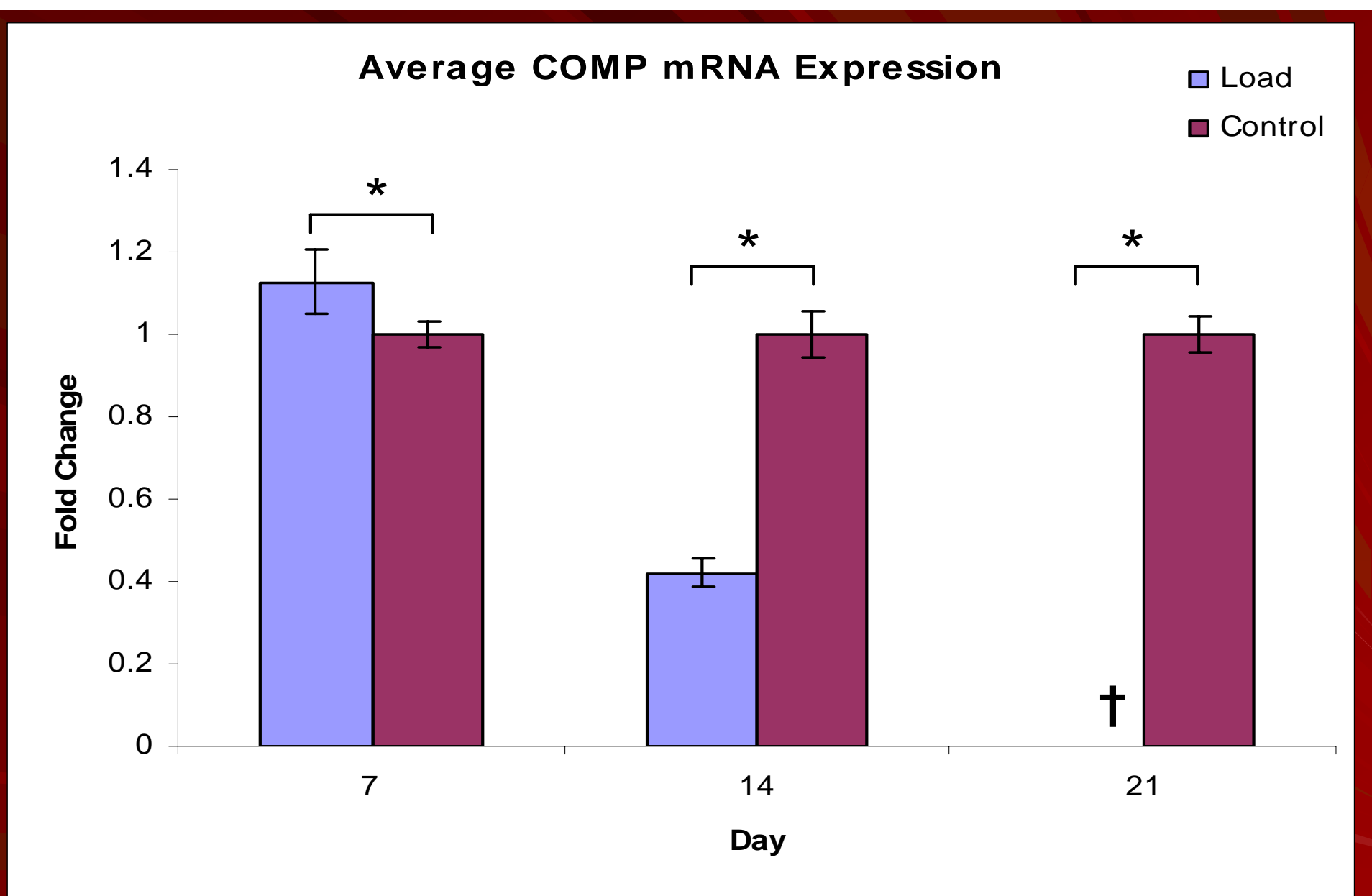


Figure 3: Average COMP mRNA expression (* indicates $p < 0.05$ and † indicates no data due to low GAPDH values)

Conclusions

- Upregulation in day 7 of Sox9, aggrecan and COMP suggests early chondrogenesis
- Previous experiments with hMSCs demonstrated a similar upregulation in Sox9 early on suggesting CHP alone is capable of inducing chondrogenesis
- GAPDH differences in loaded and unloaded at day 21 suggest decrease in cell viability
 - Not because of deficient diffusion of growth medium
 - Cells may not be able to handle CHP
 - Agarose matrix may not be good for CHP alone^{9,10}
 - Plan to run experiment with hMSCs and hASCs together to measure their viability and investigate new matrix material

Conclusions Continued

- A larger upregulation is seen in the previous bone marrow derived hMSCs experiment at day 4⁸
 - Mechanical loading upregulates factors faster and we may need to analyze them sooner than day 7
 - Plan to run experiments to analyze earlier time points

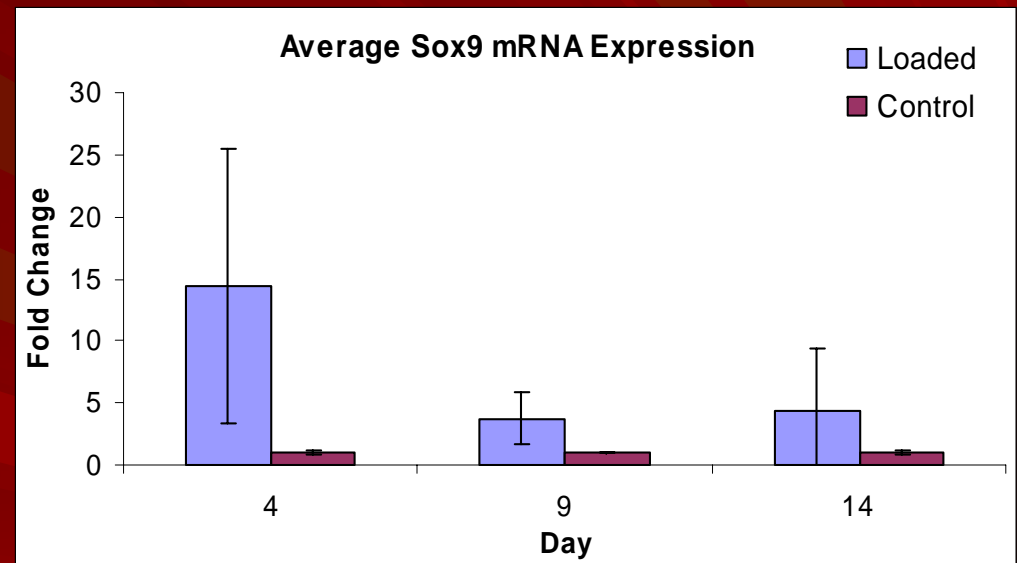


Figure 1: Average Sox9 mRNA expression for bone marrow derived hMSCs (* indicates $p < 0.05$)

Thank You

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